

435/69.1
Schmickel
1 of 15

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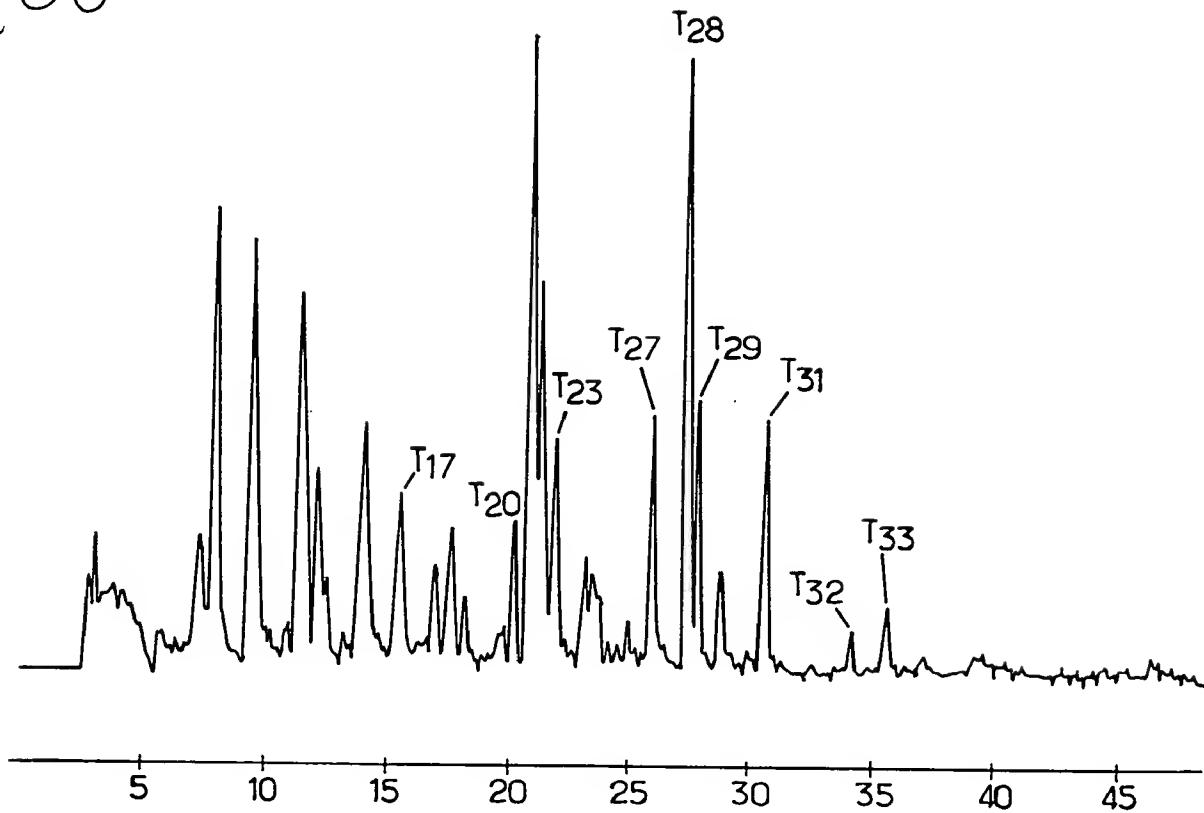


FIG. 1

Elution profile by measurement of the optical density at 218 nm
of the product of tryptic digestion of urate oxidase

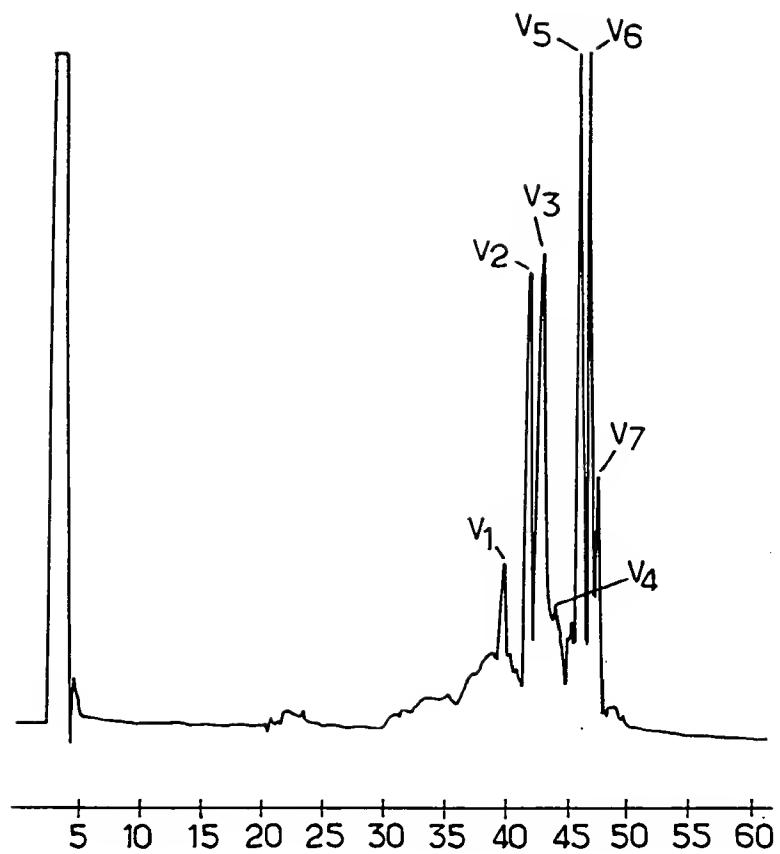


FIG. 2

Elution profile by measurement of the optical density at 218 nm
of the product of digestion of urate oxidase with protease V8

PEER HAM C 1 0721/09 5:05 02

1 AACCCCTCACTGCCCTCTCATTCCTCGG GTGCCCGATCCTCAATCCAACCTGGTACA
 61 TACTTCTCCAACTCTGTATATCCTTC ATATTCCATACTACAAGATGTCCGGAGTA 120
 121 AAGGAGCCGCTACGGCAAGGACAATGTC CGCGTCTACAGGTTCACAGGACGGAG 180
 181 ACCGGTGTCAAGACGGTGTACGGATGACC GTCTGTGCTTCTGGAGGTGAGATTGAG 240
 241 ACCCTTACACCAAGGCCGACACAGGGTC ATTGTGCAACCGACTCCATTAAAGAACACC 300
 301 ATTACATCACCGCCAAAGCAGAACCCCGT ACTCCCTCCGGAGCTGTTGGCTCCATCCTG 360
 361 GGCACACACATTGAGAAGTACAACCAAC ATCCATGCCGCTCACGTCAACATTTGTC 420
 421 CACCGCTGGACCCGGATGGACATTGACGGC AAGCCACACCCCTCACTCCCTCATCCGGAC 480
 481 AGCGAGGAGAAGGGAAATGTGCAAGGGAC ^{A*} GTGGTGAAGGGCATCGATAATCAAG 540
 541 TCGTCTCTGTCCGGCCTGACCCGGTGAAG AGCACCAACCTCGCAGTTCTGGGCTCCTG 600
 601 CGTGACCGAGTACACCAACTAAGGAGACC TGGGACCCGTATCTCTGAGCACCGACGTCGAT 660
 661 GCOACTTGGCAGTGGAGAATTCAAGTGG CTCCAGGAGGTCTCGCTGACGTGCTAAAG 720
 721 TTCGATGCTACCTGGCCACTGCTCGCGAG GTCACACTGAAAGACTTTTCGCTGAAGATAAC 780
 781 AGTGGCCAGGGTGCAGGCCCACTATGTCAG ATGGCAGAGCAAAATCCTGGCGGCCAGCAG 840
 841 CTGATCGAGACTGTGAGTACTCGTTGCCCT AACAAAGGACTATTTCGAATAATGACCTGAGC 900
 901 ^{6*} TGGCACAAGGGCCCTCAAAACACCGGCAAG AACGCGCAGGGTCTCGCTCAGTCGGAC 960
 961 CCCAACGGTCTGTATCAAGTGTACCGTCGGC CGGTCTCTCTGGAAAGTCTAAATTGTAACC 1020
 1021 AACATGATTCTCACGTTCGGAGTTCCAA GGCAACTGTATATAGTCTGGATAGGGTA 1080
 1081 TAGCATTCACTTCACTTACTTCCA AAAAAAAA... .

FIG. 3

Nucleotide sequence of clone 9C and of part of clone 9A

↓ : start of clone 9A

109 ATG TCC GCA GTAAAGCAGCCGGCTACGGC AAGGACAATGTCGCCGTCTACAAAGCTTCAC 168
 1 Met Ser Ala Val Lys Arg Tyr Gly Lys Asp Asn Val Arg Val Tyr Lys Val His

 169 AAG GAC GAG ACC CGGTGTCAGACGGGT TAC GAG ATG AAC CGT CTC GTG CCT TCT GGAG 228
 21 Lys Asp Glu Lys Thr Gly Val Glu Thr Val Ile Val Tyr Glu Met Thr Val Cys Val Leu Glu 40

229 GGT GAG ATT GAG ACC TCT TAC ACC AAG GCC GACA AAC AGCGT CATT GTG CA ACC GACT CC 288
 41 Gly Glu Ile Glu Thr Ser Tyr Thr Lys Ala Asp Asn Ser Val Ile Val Ala Thr Asp Ser 60

289 ATT A GAA ACC CATT ACAT CAC CGCC AAG CAG AAC ACC CGT TACT CCT CCC GAG CCT GTC 348
 61 Ile Lys Asn Thr Ile Tyr Thr Ala Lys Glu Asn Pro Val Thr Pro Pro Glu Leu Phe 80

349 GGCTCCATCCTGGCACACACTTCAATTGAG AAGTACAACCACATCCATGCCGCTCACGTC 408
 81 Gly Ser Ile Leu Glu Thr His Phe Ile Glu Lys Tyr Asn His Ile His Ala Ala Lys Val 100

409 AAC ATT GTC TGT GCC ACC CGCT GGAC CCGGATG GAC ATT GAC GGC AAG CCAC ACC CTCA CTCC 468
 101 Asn Ile Val Cys His Arg Trp Thr Arg Met Asp Ile Asp Gly Lys Pro His Pro His Ser 120

469 TTCA TCC CGG ACAG CGG GAG AAG CGGA AT GTG CAG CGT GCG GAG CCA ACT CGC AGG GGC 528
 121 Phe Ile Arg Asp Ser Glu Ile Lys Arg Asn Val Gln Val Asp Val Val Glu Gly Lys Gly 140

529 ATCG AT ATCAA GTC CGT CTC TGT CGT CCG GGC CTC ACC CGT GCT GAG GAG CAC CA ACT CGC AGG GGC 588
 141 Ile Asp Ile Lys Ser Ser Leu Ser Gly Leu Thr Val Ile Lys Ser Thr Asn Ser Gln Phe ^{T17} T31 160

589 TGG GGCTTCCTGGCTGACCGAGTACACCACA CTTAAGGAGAGACTGGCACCGGTATCCTGAGC 648
 161 Trp Gly Phe Ile Arg Asp Glu Ile Tyr Thr Thr Leu Lys Glu Thr Trp Asp Asp Arg Ile Leu Ser 180

649 ACC GAC GTG CGAT GCG CACT TGG CAG TGG AAG AATTTCAGTGGACTCCAGGAGGTC CGCTCG 708
 181 Thr Asp Val Asp Ile Asp Thr Trp Glu Ile Trp Lys Asn Phe Ser Gly Leu Glu Ile Val Arg Ser 200

FIG. 4 (cont. next page)

709	CACGTGCCTAAGTTCGATGCTACCTGGCC	ACTGCTCGGAGGCTCACTCTGAAAGCACTTT	768
201	<u>His</u> <u>Val</u> <u>Pro</u> <u>Ly</u> <u>sPhe</u> <u>Asp</u> <u>Ala</u> <u>Thr</u> <u>Trp</u> <u>Ala</u>	ThrAlaArgGluValThrLeuLysThrPhe	220
	→ T23	←	— — — —
769	GCTGAAGATAACAGTGCCAGGGTGCAGGCC	ACTATGTCACAGATGGCAGAGCAATCCTG	828
221	<u>Ala</u> <u>Gl</u> <u>u</u> <u>sPhe</u> <u>Asp</u> <u>Asn</u> <u>Ser</u> <u>Ala</u> <u>Ser</u> <u>Ala</u> <u>Ala</u>	ThrMetTyrLysMetAlaGluGlnIleLeu	240
	— — — —	— — — —	— — — —
829	GCGGCCAGCAGCTGATCGAGACTGCGAG	TACTCGTGCCTAACAAAGCACTATTTCGAA	888
241	<u>Ala</u> <u>Arg</u> <u>Gln</u> <u>Gln</u> <u>Leu</u> <u>Ile</u> <u>Gl</u> <u>u</u> <u>Thr</u> <u>Val</u> <u>Glu</u>	TyrSerLeuProAsnLysHistYrPheGlu	260
	→ V2	→ V2	→ T29/
889	ATCGACCTGAGCTGGCACAAAGGCCCTCCAA	AACACCGGCCAACGAAACGCCAGGGTCTTCGCT	948
261	<u>Ile</u> <u>Asp</u> <u>Leu</u> <u>sPhe</u> <u>Trp</u> <u>His</u> <u>Lys</u> <u>Gly</u> <u>Leu</u> <u>Gln</u>	AsnThrGlyLysAsnAlaGluValAlpheAla	280
	→ T27	→ V1	— — — —
949	CCTCAGTGGACCCCAACGGTCTGATCAG	TGTACCGTGGCCGGTCTCTGAAAGTCT	1008
281	<u>Pro</u> <u>Gln</u> <u>Ser</u> <u>Asp</u> <u>Pro</u> <u>Asn</u> <u>Gly</u> <u>Leu</u> <u>Ile</u> <u>Lys</u>	CysThrValGlyArgSerSerLeuLysSer	300
1009	AAATTGAA		
301	LysLeuEnd		

FIG. 4 (contd.)

DNA sequence opened by ATG in position 109 in Figure 3 and polypeptide coded for.

The sequenced peptides obtained by hydrolysis of *A. flavus* urate oxidase with trypsin and protease V8 are shown by arrows opposite the polypeptide coded for, according to

→ : tryptic peptide
 → → : peptide obtained by hydrolysis with
 protease V8.

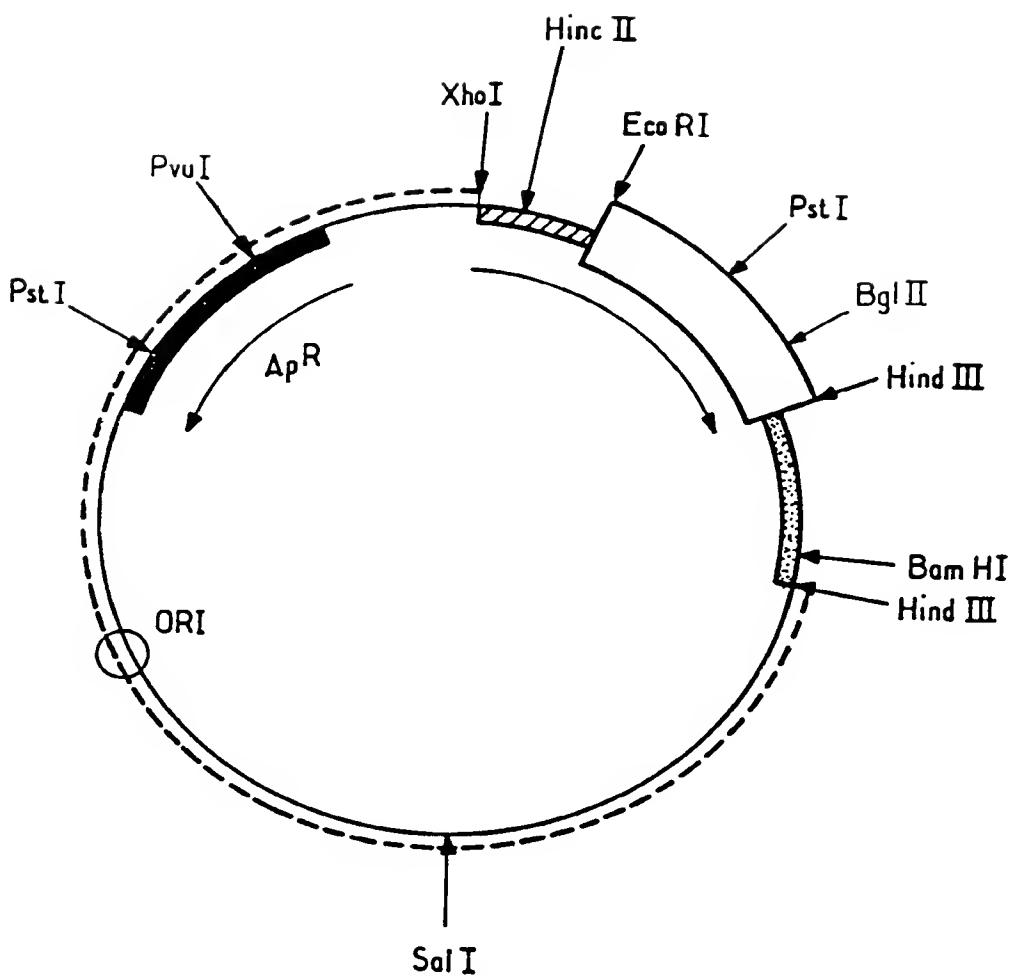


FIG. 5

Plasmid *p* 163,1

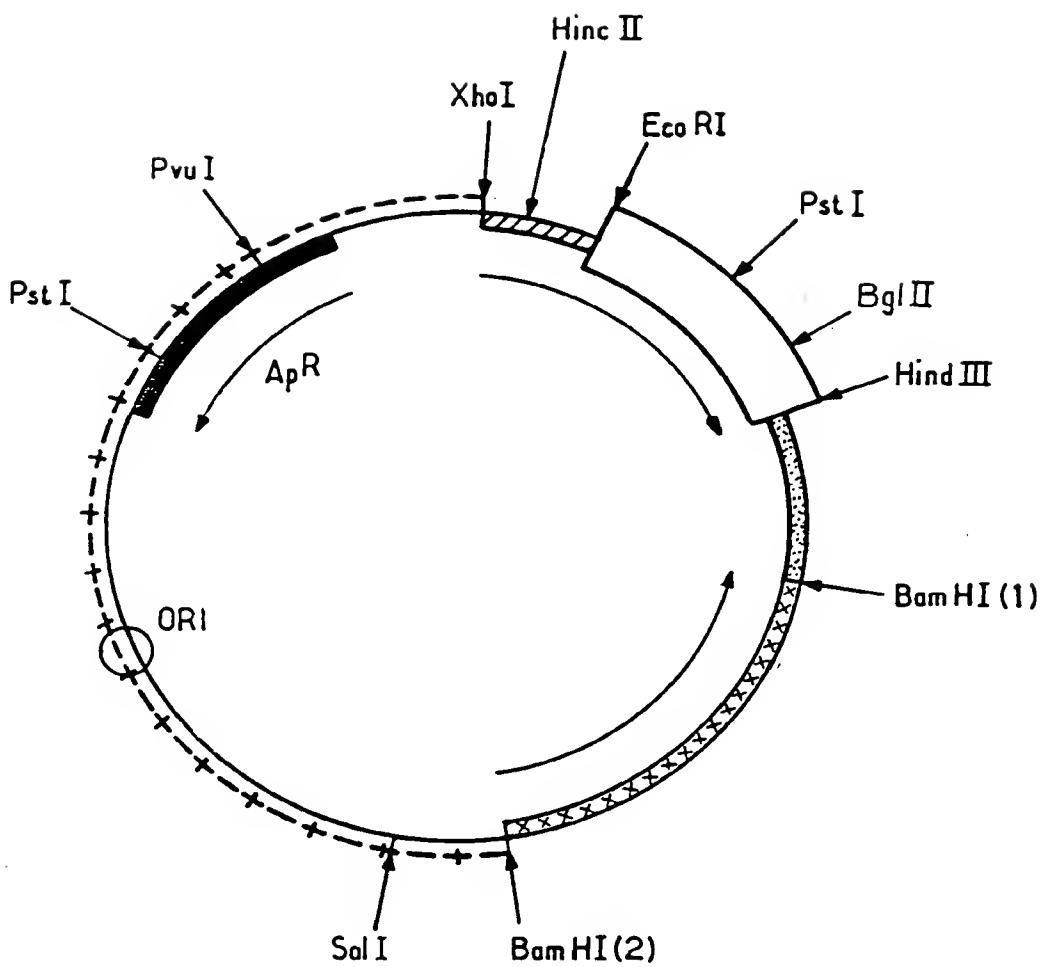


FIG. 6

Plasmid p 160

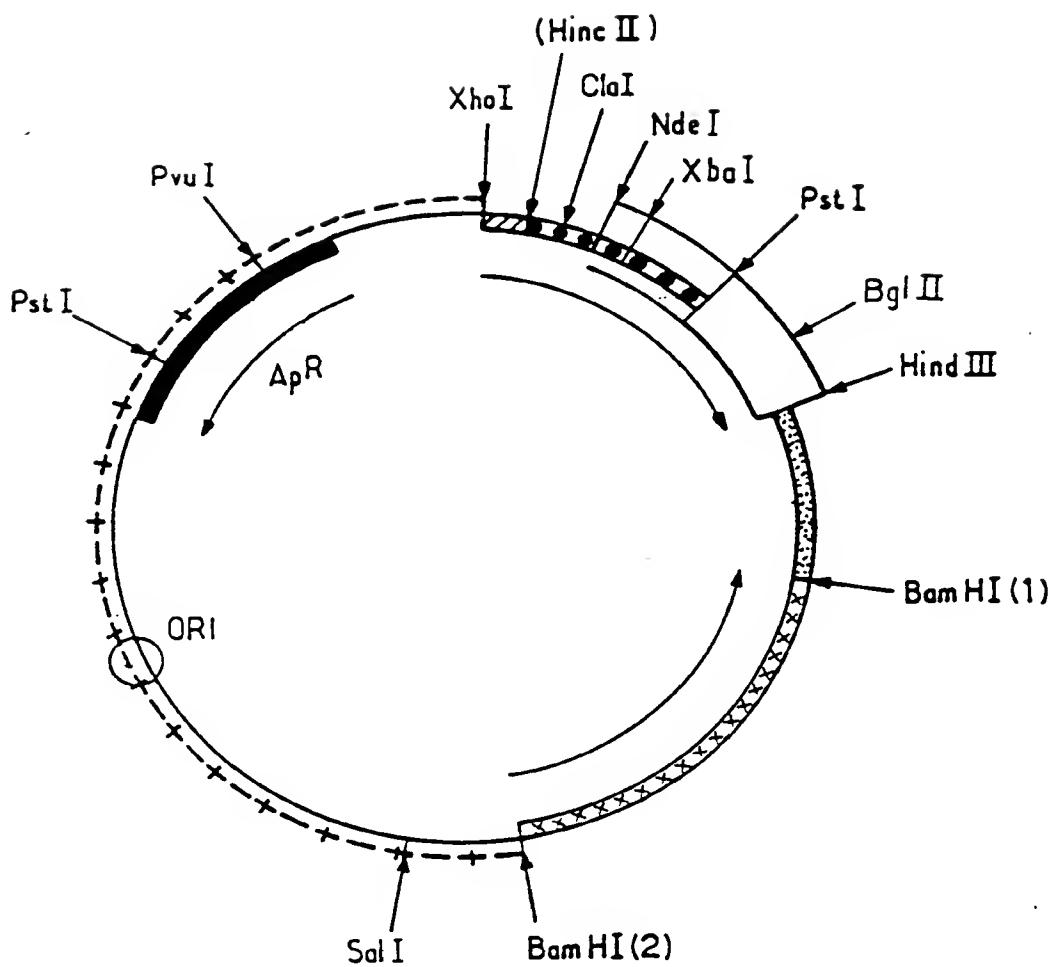


FIG. 7

Plasmid p 373,2

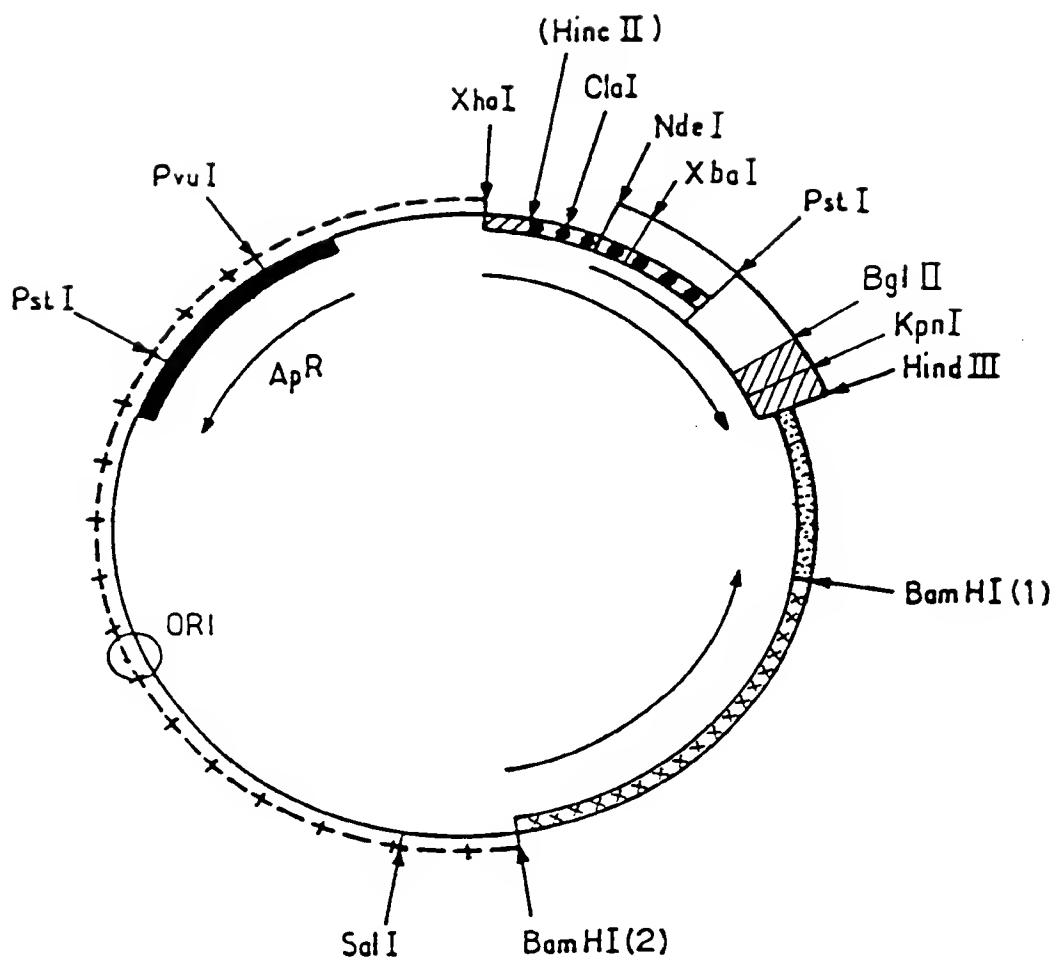


FIG.8

Plasmid p 462

17/659408

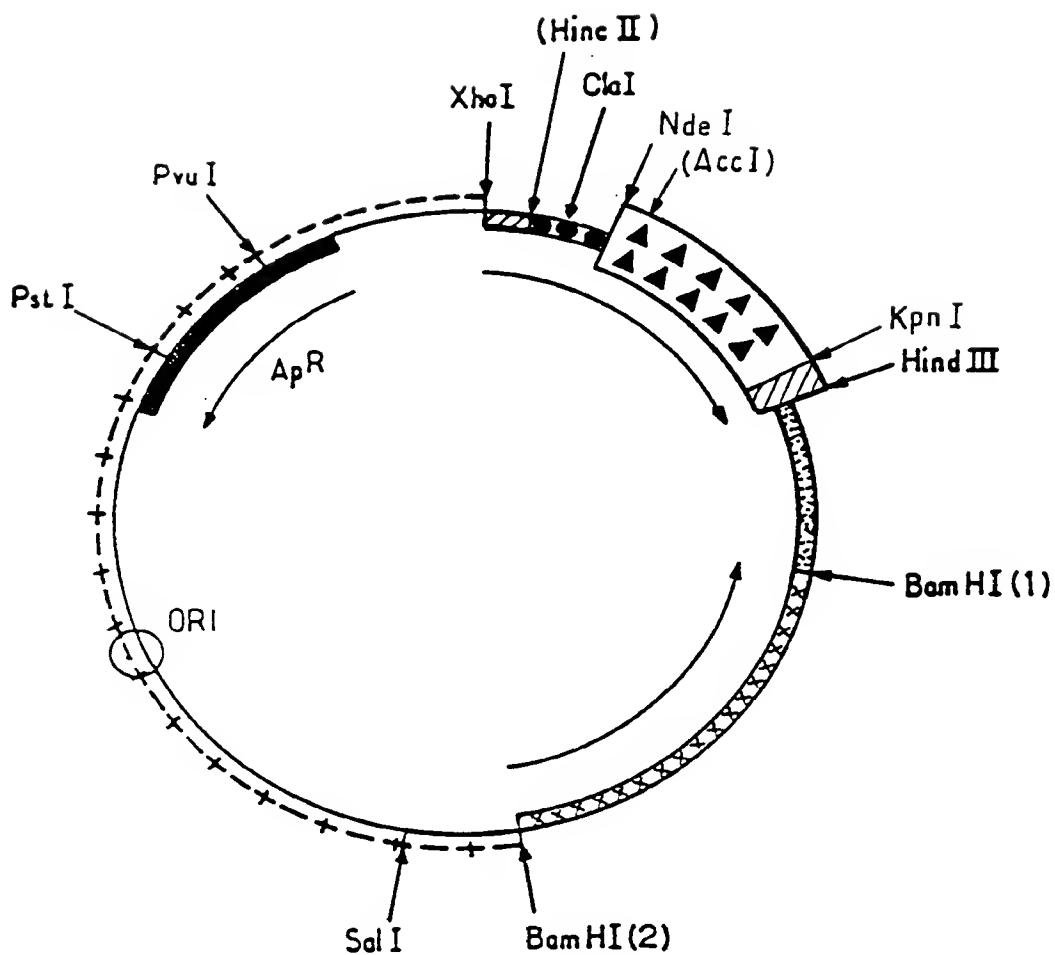


FIG. 9

Plasmid p466

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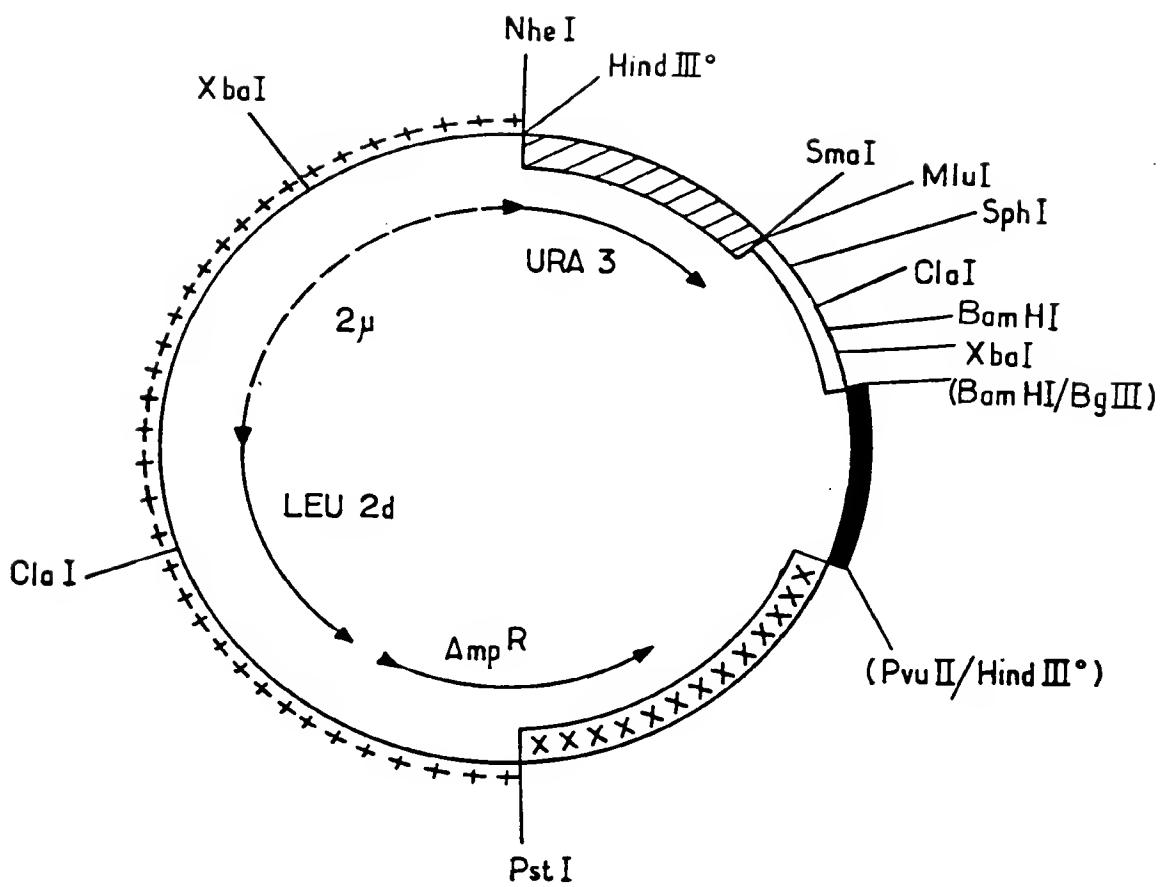


FIG.10

Plasmid pEMR 414

W/659408

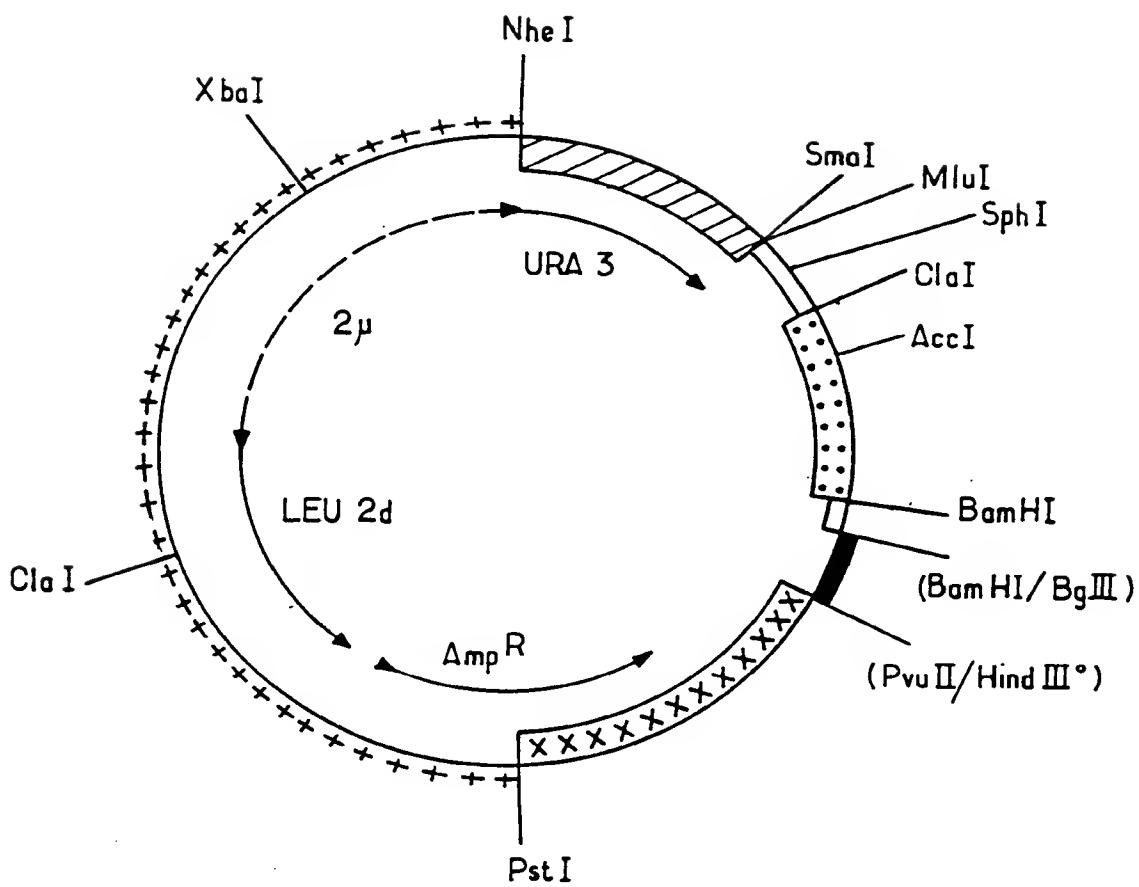


FIG.11

Plasmid pEMR 469

17/659408

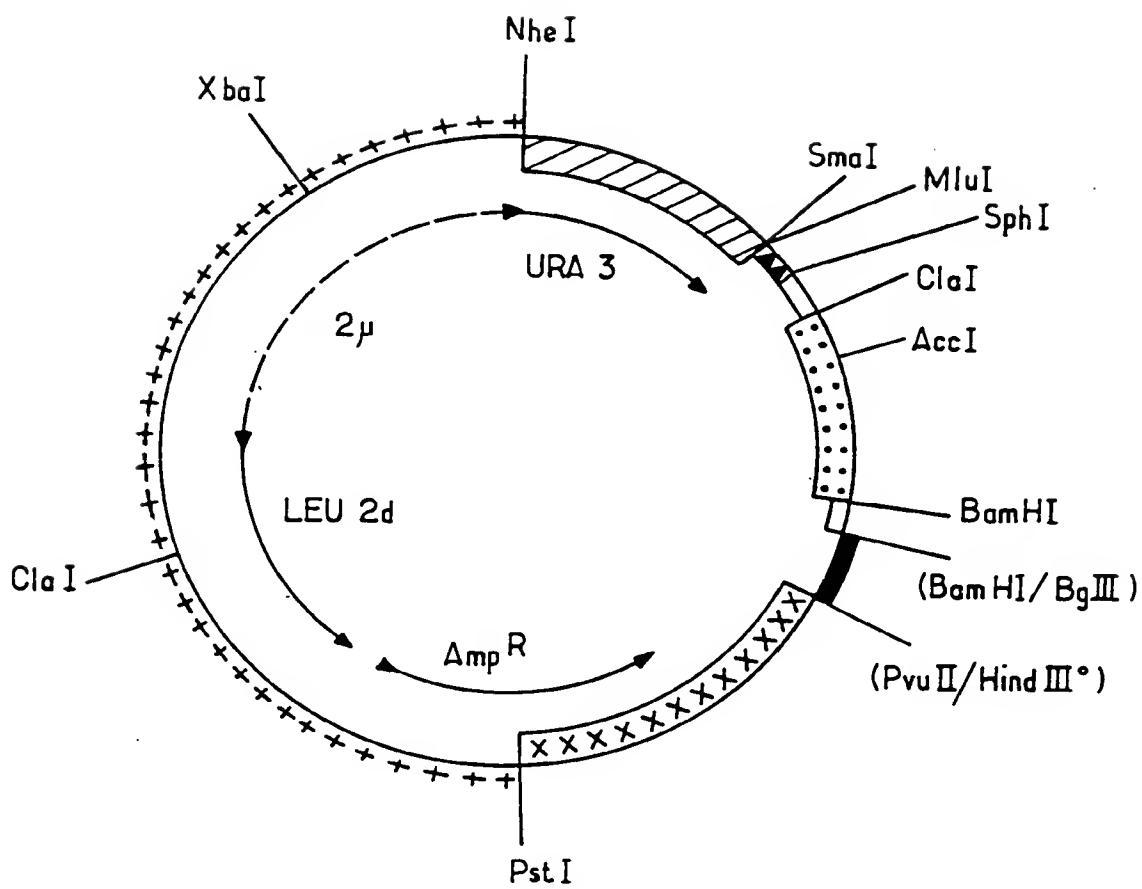


FIG.12

Plasmid pEMR 473

7/659408

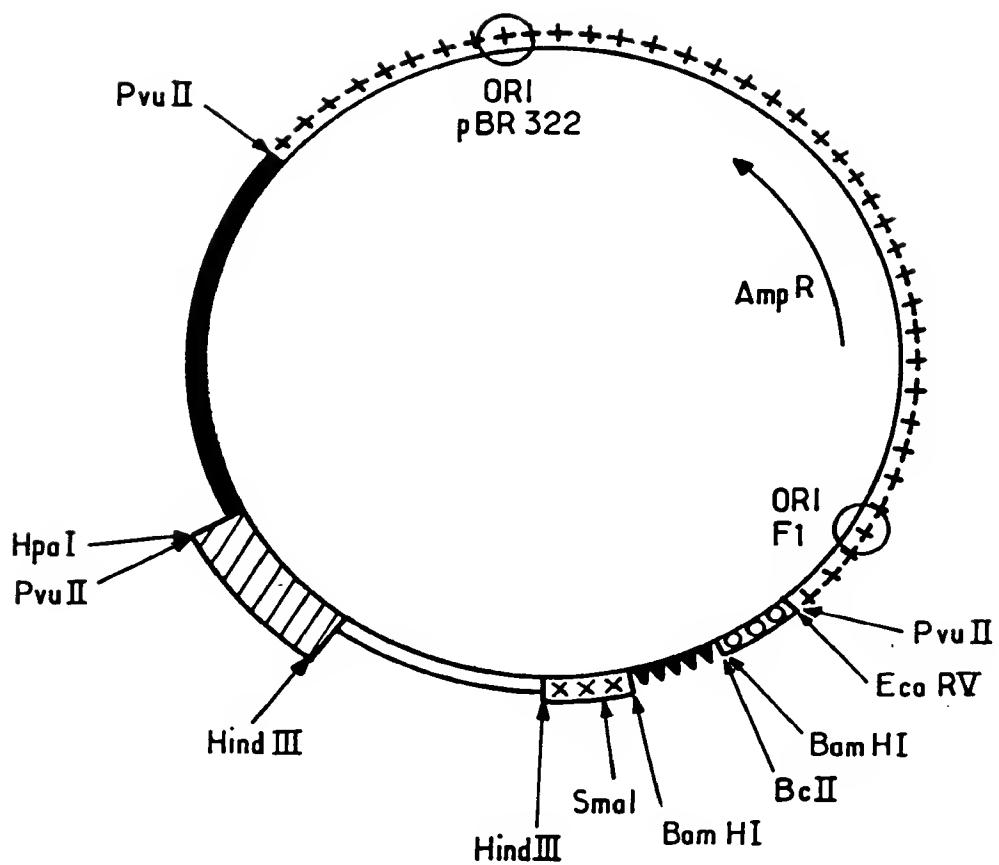


FIG.13

Plasmid PSE 1

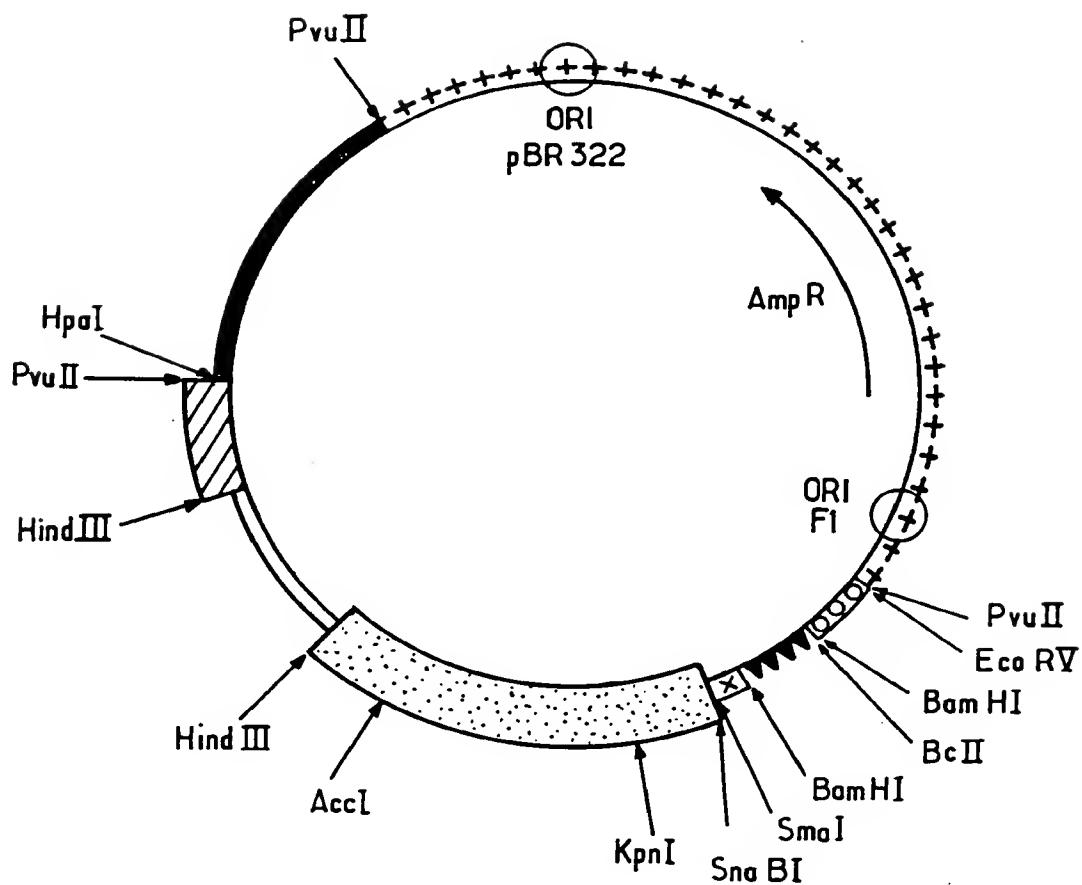


FIG.14

Plasmid pSV860